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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

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Applicant's or agent's file reference BN 46 PCT	**FOR FURTHER ACTION**	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/EP 03/05047	International filing date *(day/month/year)* 14.05.2003	Priority date *(day/month/year)* 16.05.2002
International Patent Classification (IPC) or both national classification and IPC C12N15/863, C07K14/18, A61K39/12		
Applicant BAVARIAN NORDIC A/S et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 6 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 4 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
04.12.2003	12.08.2004
Name and mailing address of the international preliminary examining authority: European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized Officer Zellner, E Telephone No. +49 89 2399-8427

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP 03/05047

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-45 as originally filed

Sequence listings part of the description, Pages

1-5 as originally filed

Claims, Numbers

1-26 received on 20.03.2004 with letter of 18.03.2004

Drawings, Sheets

1/19-19/19 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

   These elements were available or furnished to this Authority in the following language: , which is:

   ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).

   ☐ the language of publication of the international application (under Rule 48.3(b)).

   ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

   ☒ contained in the international application in written form.

   ☒ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☐ furnished subsequently to this Authority in computer readable form.

   ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

   ☐ the description, pages:

   ☐ the claims, Nos.:

   ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-21,24-26
	No:	Claims	22,23
Inventive step (IS)	Yes:	Claims	4-10,11,13
	No:	Claims	1-3,12,14-26
Industrial applicability (IA)	Yes:	Claims	1-26
	No:	Claims	

2. Citations and explanations

   see separate sheet

D1: US-A-5 338 683 (PAOLETTI ENZO) 16 August 1994 (1994-08-16)
D2: WO 98 13500 A (SUTTER GERD ;ERFLE VOLKER (DE); GSF FORSCHUNGSZENTRUM UMWELT (DE);) 2 April 1998 (1998-04-02)
D3: US-A-5 494 807 (RIVIERE MICHEL ET AL) 27 February 1996 (1996-02-27)
D4: HOWLEY P M ET AL: 'A functional measles virus replication and transcription machinery encoded by the vaccinia virus genome.' JOURNAL OF VIROLOGICAL METHODS. NETHERLANDS APR 1999, vol. 79, no. 1, April 1999 (1999-04), pages 65-74, XP002257129 ISSN: 0166-0934
D5: WO 02 18585 A (KOVACS GERALD R ;KOWALSKI JACEK (US); VASILAKIS NIKOS (US); ZAMB T) 7 March 2002 (2002-03-07)

1. Novelty

US5338683 (D1) describes a recombinant poxvirus such as a vaccinia virus containing foreign DNA from herpes virus. At least two or three glycoproteins of herpes virus glycoproteins are inserted at two different sites of the vaccinia viral vector and expressed (Examples 1, column 14, lines 40-68, column 15, lines 60, column 17, line 20-46; Example 2, column 19 lines 7-27; Example 3). A vaccine is produced.
As glycoproteins from herpes virus are to a certain extent homologous the vaccine vector constructs of D1 fall under the definition of Claim 22 and 23 (Examples 4-6). Thus Claims 22 and 23 are not novel.

2. Inventive step

2.1 WO9813500 (D2, page 5) represents the closest prior art document describing a recombinant MVA virus expressing dengue virus antigens such as preM or prM antigens inserted into one insertion site. Dengue Virus antigens from four different serotypes (serotype 1, 2, 3 and 4) are inserted into one site. In addition, the antigens from Dengue Virus serotypes are of different type such as preM,E or NS1 being not identical (D2, Claim 3 and Examples).
Thus in difference, to said document **different insertion sites** for each inserted gene are selected in the present application and the foreign genes inserted are **identical** to a certain extent such as 66.5-72.9% (description page 9).

The problem of the present application is the provision of an improved poxvirus vector. The solution is provided by the insertion of at least two genes being identical to a certain extent as defined above such as **PrM** gene of **Dengue virus** and being derived

from at least two different serotypes into **different insertion** sites such as intergenic regions of poxviral vectors such as **Modified Vaccinia Ankara virus (MVA)** in order to obtain a stable multivalent vaccine against different serotypes.
However, in the present application it is not shown that the problem has indeed been solved over the whole area claimed. In other words it has not been shown that indeed any multivalent stable poxviral vector carrying any desired gene of any organism can be made by the present poxviral vectors. It has not even been demonstrated that effective vaccines against dengue viruses can be made.
Thus the problem is reduced to the provision of an alternative poxviral vector having no particular effect. Such vectors are obviously derivable from D1, D4 (HOWLEY et al J of Virl. 79, page 66, left column, paragraph 1; or D5 WO0218585 Fig. 3). All of said documents suggest poxviral vectors with different insertion sites. Thus the skilled person would also use different insertion sites for homologous genes from different serotypes. The contribution of the present application is thus the selection of a specific vector from a large number of possible solutions. In order to be inventive, such a selection must not be arbitrary but must be justified by the technical purpose, i.e. a hitherto unknown unexpected effect, caused by those technical features which distinguish the claimed vectors from the numerous other ones.

Due to the absence of any unexpected function or technical effect of the general claims, the present selection amounts to nothing more than an arbitrary selection. Consequently, the claimed vectors of Claims 1-3,12, 14-21 and derived Claims 22-26 are considered to lack an inventive step, since the problem has not been solved for the claimed generic subject-matter.

2.2 The combination of Claims 4 to 10 and 13 and dependent claims thereof such as claim 11 do involve an inventive step. The problem has been solved in the present application (Examples). The solution i.e. the insertion of at least two PrM genes of at least two different serotypes into two different insertion sites of MVA is not obviously derivable form the prior art. As pointed out in the description of the present application (page 7, paragraph) a mixture of recombinant viruses has been used in the prior art. One viral construct for insertion of identical genes of different serotypes has not been suggested.

Additionally, it is noted that the wording "homologous" is absolutely vague and open for interpretation (Claims 1,2,3 and 22). A more suitable definition is "**identitiy**" such as

used in the description (page 9, line 6-7).
Claims 1-5 do not describe all the essential features needed to unambiguously define the invention. As pointed out above only a combination of Claims 4-10 chacterieses all the essentail technical features (Artciles 5 and 6 PCT).

For the assessment of the present claims 19,25 and 26 on the question whether they are industrially applicable, no unified criteria exist in the PCT Contracting States. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject-matter of claims to the use of a compound in medical treatment, but may allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

C L A I M S

1. A recombinant poxvirus comprising at least two homologous foreign genes, wherein each of said genes is inserted into a different insertion site of the viral genome.

2. The recombinant poxvirus according to claim 1, wherein the genes have a homology of at least 50%.

3. A recombinant poxvirus comprising at least two homologous foreign genes, said genes having a homology of at least 60%.

4. The recombinant poxvirus according to claim 2 or 3, wherein the genes have a homology of 65-75%.

5. The recombinant poxvirus according to claims 1 to 4, wherein the genes are derived from a flavivirus.

6. The recombinant poxvirus according to claim 5, wherein the flavivirus is a Dengue virus.

7. The recombinant poxvirus according to claim 5 or 6, wherein the genes are at least two homologous genes derived from at least two different serotypes of the virus.

8. The recombinant poxvirus according to claims 5 to 7, wherein the genes are at least two PrM genes.

9. The recombinant poxvirus according to claims 5 to 8, wherein the genes are 4 PrM genes.

10. The recombinant poxvirus according to claims 1 to 9, wherein the poxvirus is a Vaccinia virus.

11. The recombinant poxvirus according to claim 10, wherein the Vaccinia virus is a Modified Vaccinia Ankara (MVA) virus.

12. The recombinant poxvirus according claim 11, wherein the MVA is MVA-BN deposited at the European Collection of Animal Cell Cultures (ECACC) under number V00083008.

13. The recombinant poxvirus according to claims 1 to 12, wherein the poxvirus is replication deficient or replication incompetent in mammalian cells, including human cells.

14. The recombinant poxvirus according to claims 1 to 13, wherein the genes are inserted into a naturally occurring deletion site and/or into an intergenic region of the poxviral genome.

15. The recombinant poxvirus according claims 1 to 14 as medicament or vaccine.

16. A vaccine comprising the recombinant poxvirus according to any of the claims 1 to 14.

17. A pharmaceutical composition comprising the recombinant poxvirus according to any of the claims 1 to 14 and a pharmaceutically acceptable carrier, diluent, adjuvant and/or additive.

18. The recombinant poxvirus according to any of the claims 1 to 14, the vaccine according to claim 16 or the composition according to claim 17 for affecting, preferably inducing, an immune response of a living animal, including a human.

19. Use of the recombinant poxvirus according to any of the claims 1 to 14 for the preparation of a medicament.

20. A method for affecting, preferably inducing, an immune response in a living animal, including a human, comprising administering a therapeutically effective amount of the recombinant poxvirus according to any of the claims 1 to 14, the vaccine according to claim 16 or the composition according to claim 17 to the animal or human to be treated.

21. A cell comprising the recombinant poxvirus according to claims 1 to 14.

22. A method for producing a recombinant poxvirus according to claims 1 to 14 comprising the steps of

- infecting a cell with a poxvirus;
- transfecting the infected cell with a first vector construct comprising a gene being heterologous to the poxviral genome, and a genomic poxvirus sequence capable of directing the integration of the heterologous gene into an insertion site of the poxviral genome;
- identifying, isolating and, optionally, purifying the generated recombinant poxvirus;
- repeating the above steps by using the recombinant poxvirus obtained from previous steps for infecting the cell and an additional vector construct comprising a further gene being heterologous to the poxviral genome and homologous to the gene of the first vector construct.

23. A kit comprising

- two or more vector constructs, each construct comprising a gene under transcriptional control of a poxviral expression control element, wherein the genes included in the different vectors are homologous genes, and wherein each gene is flanked by a poxviral DNA sequence

capable of directing the integration of the gene into a poxviral genome, and

- means for identifying and/or selecting recombinant poxviruses, which have incorporated said homologous genes into their genome.

24. The kit according to claim 23, wherein each homologous gene is flanked by a poxviral DNA sequence capable of directing the integration of said homologous gene of each vector construct into a different insertion site of the poxviral genome.

25. A DNA sequence derived from or homologous to the recombinant poxviral genome of the recombinant poxvirus according to claims 1 to 14, wherein said DNA sequence comprises at least two homologous genes and at least part of the sequences of the poxviral genome.

26. A method for detecting cells infected with the recombinant poxvirus according to claims 1 to 14, said method comprising administering the DNA sequence according to claim 25 to said cells.

27. A method for identifying the recombinant poxvirus according to claims 1 to 14, said method comprising administering the DNA sequence according to claim 25 to said virus.